FORM PTO-1390 **U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE**
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: 9609 V/vmf

U.S. APPLN. NO (if known, see 37 CFR 1.5) 09/980485

INTERNATIONAL APPLICATION NO.: PCT/EP00/05066	INTERNATIONAL FILING DATE: 02 JUNE 2000 (02.06.00)	PRIORITY DATE CLAIMED: 03 JUNE 1999 (03.06.99)

TITLE OF INVENTION: 10-FORMYLTETRAHYDROFOLATE DEHYDROGENASE AS THERAPEUTICAL AGENT

APPLICANT(S) FOR DO/EO/US: Alberto BARTORELLI

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [X] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. [X] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [X] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] has been transmitted by the International Bureau. (see attached copy of PCT/IB/308)
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [ ] A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. [ ] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been transmitted by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [ ] have not been made and will not be made.
8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [ ] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] A translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Item 11. to 16. below concern document(s) or information included:

11. [X] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [X] A **FIRST** preliminary amendment.
    [ ] A **SECOND** or **SUBSEQUENT** preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [X] Other items or information: INTERNATIONAL PRELIMINARY EXAMINATION REPORT (PCT/IPEA/409), INTERNATIONAL SEARCH REPORT (PCT/ISA/210), APPLICATION DATA SHEET, ABSTRACT

U.S. APPLICATION NO. (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NO.
09/980485	PCT/EP00/05066	9609 V/vmf

	CALCULATIONS PTO USE ONLY

17. [X] The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):
Neither international preliminary examination fee (37 CFR1.482) nor international search fee (37 CFR1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO ..... $ 1,040.00

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO ..... $ 890.00

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO ..... $ 740.00

International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) ..... $ 710.00

International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) ..... $ 100.00

			ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 890.00
Surcharge of $130.00 for furnishing the oath or declaration later than 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				$ 130.00
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	$
Total claims	3 - 20 =	0	X $18.00	$
Independent claims	3 - 3 =	0	X $84.00	$
MULTIPLE DEPENDENT CLAIMS(S) (if applicable)			+ $280.00	$
			TOTAL OF ABOVE CALCULATIONS =	$ 1020.00
Reduction of ½ for filing by small entity, if applicable. Applicant claims Small Entity Status under 37 CFR 1.27. +				$
			SUBTOTAL =	$ 1020.00
Processing fee of $130 for furnishing the English translation later than months from the earliest claimed priority date (37 CFR1.492(f)).				$
			TOTAL NATIONAL FEE =	$ 1020.00
Fee for recording the enclosed assignment (37 CFR1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +				$
			TOTAL FEES ENCLOSED =	$ 1020.00
			Amount to be refunded:	
			charged:	

a. [X] A check in the amount of $ **1020.00** to cover the above fees is enclosed.

b. [ ] Please charge my Deposit Account No. **25-0120** in the amount of $ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required by 37 CFR 1.16 and 1.17, or credit any overpayment to Deposit Account No. **25-0120**. A duplicate copy of this sheet is enclosed.

SEND ALL CORRESPONDENCE TO:

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December 3, 2001

By Benoît Castel
Benoît Castel
Attorney for Applicant
Registration No. 35,041

PATENTS

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Alberto BARTORELLI

Serial No. (unknown)

Filed herewith

Box Non-fee Amendment

GROUP

Examiner

10-FORMYLTETRAHYDROFOLATE DEHYDROGENASE AS THERAPEUTICAL AGENT

PRELIMINARY AMENDMENT

Commissioner for Patents

Washington, D.C. 20231

Sir:

Prior to the first Official Action and calculation of the filing fee, please amend the above-identified application as follows:

IN THE ABSTRACT:

Please delete the abstract as originally filed which appears on the cover page of the Published Application. Add new abstract enclosed herewith on a separate sheet.

Respectfully submitted,

YOUNG & THOMPSON

By Benoît Castel

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December 3, 2001

10-FORMYLTETRAHYDROFOLATE DEHYDROGENASE AS THERAPEUTICAL AGENT

The present invention relates to the use of 10-formyltetrahydrofolate dehydrogenase as therapeutical agent, in particular as cytotoxic and antitumour agent.

10-Formyltetrahydrofolate dehydrogenase is an enzyme present in the liver and in the nervous system of mammals. No therapeutical use for such enzyme has been disclosed up to now.

cDNA from rat 10-formyltetrahydrofolate dehydrogenase has been disclosed in J. Biol. Chem. 266(8), 4965-4973, 1991, while cDNA of the same human enzyme has been disclosed more recently (Biochem. Mol. Biol. Int., 47(3), 407-415, 1999).

Furthermore, methods for the preparation of the recombinant enzyme are known from Protein Expression Purif. 6, 457-64, 1995 and Biochem. J. 306(3), 651-5, 1995.

It has now been found that mammal 10-formyltetrahydrofolate dehydrogenase is capable of inducing a marked cytotoxic response against tumour cells, when administered to tumour-bearing patients or animals.

This cytotoxicity seems to be mediated by cytotoxic antibodies to human tumour cells, particularly carcinomas and adenocarcinomas.

Cytotoxicity can be quantified in vitro on Jurkat and Kato III cells using conventional methods, based for example on the use of commercial kits such as the CDC-UK kit (Pharmaproduct). In particular, the appearance of cytotoxicity in rabbits serum was observed already after a first treatment with the enzyme (1 mg/animal in saline solution) on Jurkat and Kato III cells.

Therefore, the invention also relates to pharmaceutical compositions containing as active ingredient

an effective dose of 10-formyltetrahydrofolate dehydrogenase.

The compositions of the invention will be administered to tumour patients using the conventional administration routes for proteins and polypeptides, for example the subcutaneous or intramuscular routes. The treatment may be repeated, a treatment comprising one-two week separated administrations of doses ranging from 0.1 to 20 mg of enzyme being preferred.

Furthermore, it has surprisingly found that it is possible to induce high cytotoxicity by administering the enzyme even at very low dosages, such as 1.10^{-4} - 1.10^{-10} g, through the sublingual route, in the form of granules or drops of 1% water-alcoholic solutions or suspensions in ethanol, with concentrations of active ingredient ranging from 10^{-6} to 10^{-10} M.

10-Formyltetrahydrofolate dehydrogenase can be prepared by conventional recombinant DNA methods or it can be extracted from the liver of animals, for example from liver of bovine, ovine or swine. Goat liver proved to be a particularly abundant source of this enzyme.

The extraction process comprises the treatment of livers with solutions buffered at pH 7.4 (PBS) followed by precipitation with 15% polyethylene glycol 6000, chromatography on TSK-DEAE or DEAE-Sephacell at pH 8, elution with 0.3 M NaCl and purification on TSK SW3000.

The following example illustrates the invention in greater detail.

EXAMPLE

Extraction

50 g of goat liver are homogenized, suspended in 400 ml of PBS 0.01 M pH 7.2, stirred for 30 minutes at 4°C and centrifuged on JA14 at 14,000 RPM for 30 minutes. After that, the product is filtered with suction; then through

1.2 μm filter, finally through 0.45 μm filter.

Volume: 340 ml conc. 10.9 mg/ml.

Fractional precipitation with PEG 6000.

336 ml of the above sample are treated with 5% powder PEG 6000 (16.8 g). The whole is stirred for 1 hour at 4°C, then centrifuged on J 6 at 4,000 g for 30'.

The pellet is taken up into 61 ml of 0.03M Tris/HCl pH 8, whereas the supernatant (340 ml) is reprecipitated with 5% PEG 6000 (17 g), then 10% PEG 6000 stirring for 1 hour at 4°C.

After centrifuging on J6 at 4,000 g for 30', the pellet is taken up with 62 ml of 0.03M Tris/HCl pH 8.

The supernatant (345 ml) is treated with 5% PEG 6000 (17.25 g), then again with 5% PEG 6000, stirring for 1 hour at 4°C, then centrifuged on J6 at 4,000 g for 30'.

The supernatant is discarded, and the pellet is taken up into 200 ml of 0.03M Tris/HCl pH 8.

5% PEG pellet volume: 61 ml, conc. 9.34 mg/ml.

10% PEG pellet volume: 62 ml, conc. 13 mg/ml.

15% PEG pellet volume: 200 ml, conc. 3.38 mg/ml.

DEAE - Sephacell

About 150 ml of DEAE-S resin are equilibrated in 0.03 M Tris/HCl buffer pH 8. The resin is incubated with the 15% PEG sample for 30 minutes at room temperature + 200 ml of washing.

Leg 1: 200 ml 0.5M Tris/HCl pH 8 for 30 minutes at r.t. + 200 ml of washing.

Leg 2: 200 ml 0.03M Tris/HCl pH 8 + 0.3M NaCl for 30 minutes at r.t. + 200 ml of washing.

Leg 3: 200 ml 0.03M Tris/HCl pH 8 + 1M NaCl for 30 minutes at r.t. + 200 ml of washing.

The following samples are thereby obtained:

S.B. volume: about 400 ml conc: 294 ✓/ml.

LEG 1 volume: about 400 ml conc: 1.14 mg/ml.

LEG 2 volume: about 400 ml conc: on PM 30.

LEG 3 volume: about 400 ml conc: 137 γ/ml.

LEG 2 is conc. on PM 30 to an about 20 ml final volume, concentration of about 3.6 mg/ml.

SW3000 prep.

LEG 2 from DEAE-S obtained above is purified in prep. SW3000 prep. (10 runs, 2 ml each).

Four fractions are eluted, the second being concentrated on PM 30 and dyalised against H_2O to a final volume of about 2 ml, concentration of about 1.5 mg/ml.

CLAIMS

1. 10-Formyltetrahydrofolate dehydrogenase as therapeutical agent.

2. 10-Formyltetrahydrofolate dehydrogenase as antitumour agent.

3. The use of 10-formyltetrahydrofolate dehydrogenase for the preparation of cytotoxic and antitumour medicaments.

S 6 of V

Ref. _____

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

10-Formyltetrahydrofolate dehydrogenase as therapeutical agent

the specification of which: *(check one)*

REGULAR OR DESIGN APPLICATION

[ ] is attached hereto.

[ ] was filed on _____ as application Serial No. _____ and was amended on _____ (if applicable).

PCT FILED APPLICATION ENTERING NATIONAL STAGE

[X] was described and claimed in International application No. PCT/EP00/05066 filed on 02.06.2000 and as amended on _____ (if any).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

PRIORITY CLAIM

I hereby claim foreign priority benefits under 35 USC 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

PRIOR FOREIGN APPLICATION(S)

Country	Application Number	Date of Filing (day, month, year)	Priority Claimed
Italy	MI99A001243	03.06.1999	YES
Italy	MI99A002197	20.10.1999	YES

(Complete this part only if this is a continuing application.)

I hereby claim the benefit under 35 USC 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of 35 USC 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37 Code of Federal Regulations §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.) (Filing Date) (Status--patented, pending, abandoned)

POWER OF ATTORNEY

The undersigned hereby authorizes the U.S. attorney or agent named herein to accept and follow instructions from ____ _____ as to any action to be taken in the Patent and Trademark Office regarding this application without direct communication between the U.S. attorney or agent and the undersigned. In the event of a change in the persons from whom instructions may be taken, the U.S. attorney or agent named herein will be so notified by the undersigned.

As a named inventor, I hereby appoint the following attorney(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: **Robert J. PATCH, Reg. No. 17,355, Andrew J. PATCH, Reg. No. 32,925, Robert F. HARGEST, Reg. No. 25,590, Benoît CASTEL, Reg. No. 35,041, Eric JENSEN, Reg. No. 37,855, and Thomas W. PERKINS, Reg. No. 33,027**, c/o YOUNG & THOMPSON, Second Floor, 745 South 23rd Street, Arlington, Virginia 22202.

Address all telephone calls to Young & Thompson at 703/521-2297.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of sole or first inventor: Alberto BARTORELLI
(given name, family name)

Inventor's signature _____ Date 12.02.2002

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Full name of second joint inventor, if any:
(given name, family name)

Inventor's signature _____ Date _____

Residence: Citizenship:

Post Office Address:

Full name of third joint inventor, if any:
(given name, family name)

Inventor's signature _____ Date _____

Residence: Citizenship:

Post Office Address: